

## Efficiency of a phytoplankton-based and a bacteria-based food web in a pelagic marine system

Johnny Berglund,<sup>1</sup> Umut Müren, Ulf Båmstedt, and Agneta Andersson

Marine Ecology, Department of Ecology and Environmental Science, Umeå University, SE-901 87 Umeå, Sweden; Umeå Marine Sciences Centre, SE-910 20 Hörnefors, Sweden

### Abstract

The food web efficiency in two contrasting food webs, one phytoplankton based and one bacteria based, was studied in a mesocosm experiment using seawater from the northern Baltic Sea. Organisms included in the experiment were bacteria, phytoplankton, protozoa, and mesozooplankton (copepods). A phytoplankton-based food web was generated by incubating at a high light level with the addition of nitrogen and phosphorus (NP). A bacteria-based food web was created by adding carbon, nitrogen, and phosphorus (CNP) and incubating at a lower light level. In the CNP treatment bacteria dominated the productivity (91%), while in the NP treatment phytoplankton were dominant producers (74%). The phytoplankton community in the NP treatment was dominated by autotrophic nanoflagellates. The food web efficiency, defined as mesozooplankton productivity per basal productivity (phytoplankton + bacteria), was 22% in the phytoplankton-based food web and 2% in the bacteria-based food web. This discrepancy could be explained by 1–2 extra trophic levels in the bacteria-based food web where carbon passed through flagellates and ciliates before reaching mesozooplankton, while in the phytoplankton-based food web there was a direct pathway from phytoplankton to mesozooplankton. The results were supported by stable isotope analysis of mesozooplankton. We propose that climate change, with increased precipitation and river runoff in the Baltic Sea, might favor a bacteria-based food web and thereby reduce pelagic productivity at higher trophic levels.

In pelagic systems, phytoplankton and bacteria are the most important producers of particulate organic material from inorganic and dissolved organic sources. Bacteria are generally more important in low-productive waters, while phytoplankton are dominant in nutrient-rich waters (e.g., Gasol et al. 1997). The organic matter can be transferred to higher trophic levels through the “microbial” or the “classical” pathway depending on the size of the resource and the size ratio between the predator and prey (Azam et al. 1983; Fenchel 1988). The number of trophic steps between the producer and the top predator varies with the dominant pathway. Owing to smaller sizes of the resources and predators in bacteria-based food webs, these generally have more trophic levels than phytoplankton-based webs. Larger carbon losses can be expected in bacteria-based food webs, since about 70% of the ingested carbon is lost at each trophic level because of respiration and sloppy feeding (Straile 1997). The size structure of the food web is therefore of importance for the ecological efficiency of a system. The ecological efficiency of a food web can be defined as the ratio between the productivity at the highest trophic level and the productivity at the base level, and is

here called food web efficiency (FWE) (Rand and Stewart 1998).

The FWE is lower in oligotrophic and strongly eutrophied waters than in moderately nutrient-rich systems (e.g., in upwelling areas) (Sommer et al. 2002). The dominant producers in oligotrophic oceanic systems are picoplankton (<2–3  $\mu\text{m}$ ), which are too small to be effectively ingested by mesozooplankton (Finlay and Roff 2004; Vargas and Gonzalez 2004). Heterotrophic nanoflagellates or small ciliates may therefore constitute an additional trophic link in oligotrophic systems (Caron et al. 1999; Sherr and Sherr 2002). In moderately nutrient-rich systems, the primary productivity may be dominated by nanoplankton, which can be directly consumed by mesozooplankton (Koshikawa et al. 1999). In eutrophic systems the lower food web efficiency is explained by increased abundances of inedible or toxic algae (McCauley et al. 1999; Sommer et al. 2002). Predation defense mechanisms or dominance of disfavored prey items (e.g., filamentous phytoplankton) are quite common in such aquatic environments (Havens et al. 2000; Persson et al. 2001).

At the initial recognition of the microbial loop, the question whether it constituted a “sink” or a “link” in the transfer of energy to higher trophic levels was discussed (Azam et al. 1983; Ducklow et al. 1986). The microbial loop may be considered as a sink in its original depicted form, since the carbon derived from primary productivity is severely reduced through respiration if it is channeled through the microbes instead of transported directly to mesozooplankton (e.g., Fenchel 1988). The microbial food web is certainly a link because dissolved organic matter is transferred via the microbial loop to higher trophic levels. Furthermore, in many marine and freshwater pelagic systems, allochthonous dissolved organic carbon (ADOC)

<sup>1</sup> Corresponding author (johnny.berglund@emg.umu.se).

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is supporting bacterial growth and the pelagic food web (Rolff and Elmgren 2000; Pace et al. 2004; Sandberg et al. 2004). For example, in the northern Baltic Sea, the ADOC supply accounts for 40% of the carbon input into the food web (Sandberg et al. 2004). In addition, stable isotope analyses show that this carbon reaches mesozooplankton (Rolff and Elmgren 2000). It is evident in such systems that the microbial food web is an important link to mesozooplankton.

Despite the importance of this topic for understanding the regulating mechanisms of productivity in aquatic systems, the literature is sparse. We have only found a few studies that directly address the question of the relative efficiency of bacterial and phytoplankton pathways (Koshikawa et al. 1996; Havens et al. 2000). In these studies the transport of carbon from algae and bacteria up through the food web to mesozooplankton was studied by labeling the basal resources (bacteria and phytoplankton) with radioactive dissolved organic carbon (DOC) and dissolved inorganic carbon (Koshikawa et al. 1996; Havens et al. 2000). In general, both the algae- and bacteria-based pathways were found to be relatively inefficient. The low efficiency of the bacterial pathway was attributed to many trophic levels, while the low FWE in the algal based pathway was explained by dominance of inedible autotrophs: filamentous cyanobacteria. Blomqvist et al. (2001) added labile carbon to an oligotrophic clear-water lake. Before addition of carbon, the basal resource of this lake was dominated by green algae. After addition, the algae decreased, heterotrophic bacteria and protozoa increased, and the biomass of mesozooplankton decreased. This implies that the overall food web efficiency decreased in this lake after carbon addition. The plankton biomasses became similar to those of a closely situated humic lake, highly influenced by ADOC. This indicates that bacterial pathways have lower efficiency than phytoplankton pathways in low-productivity areas.

We experimentally studied how changes of the basal resource affected the ecological efficiency of a marine food web. A mesocosm experiment was carried out using a natural plankton community from a coastal sea area in the northern Baltic Sea. The food web comprised organisms from bacteria and phytoplankton at the lowest trophic levels to mesozooplankton (copepods) at the highest trophic level. We aimed to keep the combined productivity of the bacteria and phytoplankton at a constant level. A phytoplankton-based food web was obtained by adding inorganic N and P, and a bacteria-based food web was generated by adding organic C and inorganic N and P. We studied the effect of resource heterogeneity on the food web efficiency by measuring productivity rates, along with analyses of stable isotope ratios of nitrogen and carbon in mesozooplankton to elucidate differences in food web length and used carbon source (Post 2002).

## Materials and methods

*Experimental setup and samplings*—The experiment was performed September–October 2003 using seawater from the northern Baltic Sea (63°34'N, 19°54'E) and incubation

in eight indoor tanks (mesocosms, 400 L, diameter 1 m). Seawater was collected at 5 m depth; the salinity was 5, and the in situ temperature 12°C. No prefiltration was made. Air was gently bubbled in the mesocosms to create a well-mixed water column. Four liters, corresponding to ~1% of the total volume, was replaced daily with filtered seawater (0.22- $\mu\text{m}$  pore size polycarbonate filters). A 35- $\mu\text{m}$  mesh covered the outlet siphon to prevent mesozooplankton from leaving the mesocosms. To create a phytoplankton-based food web, nitrogen and phosphorus were added daily to four tanks (NP treatment). Added nutrients were ammonium 0.33  $\mu\text{mol L}^{-1} \text{d}^{-1}$ , nitrate 1.97  $\mu\text{mol L}^{-1} \text{d}^{-1}$ , and phosphate 0.23  $\mu\text{mol L}^{-1} \text{d}^{-1}$ . To create a bacteria-based food web, carbon, nitrogen, and phosphorus were added daily to four tanks (CNP treatment). Added carbon was glucose, 10.73  $\mu\text{mol L}^{-1} \text{d}^{-1}$ , and added nitrogen and phosphorus were similar as in the NP treatment. The C:N:P molar ratio of added nutrients was 50:10:1, in accordance to stoichiometry of bacteria (Fagerbakke et al. 1996). The experiment comprised two treatments with four replicates. Light was supplied for 12 h per day using Philips halogen (H3, 55 watt) lamps. The surface irradiance (photosynthetic active radiation) was ~100  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  in the NP treatment and ~10  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  in the CNP treatment. The length of the experiment was 31 d, and the water temperature was kept at 15°C.

Samples for enumeration of microorganisms and chlorophyll *a* (Chl *a*) were taken every third to fourth day. Primary and bacterial productivity, oxygen consumption, and mesozooplankton abundance were measured once a week. Inorganic nutrients and phytoplankton composition were measured at the start, middle, and end of the experiment. Carbon and nitrogen content and isotopic signal of mesozooplankton were analyzed at the end of the experiment.

*Analyses of samples*—Samples for heterotrophic bacteria and flagellates were preserved with 0.2- $\mu\text{m}$  filtered glutaraldehyde (1% final concentration). For analysis of bacteria, 1–3 mL water was filtered onto black 0.2- $\mu\text{m}$  25-mm polycarbonate filters (Poretics) and stained with acridine orange (0.01% final concentration). Samples were analyzed in an epifluorescence microscope (Nikon TE 300) using blue excitation light connected to an image analysis system (Blackburn et al. 1998). For analysis of flagellates, 5–10 mL water was filtered onto black 0.6- $\mu\text{m}$  polycarbonate filters (Poretics), stained with 4',6-diamidino-2-phenylindole (DAPI) according to Porter and Feig (1980), and counted with a Nikon TE 300 epifluorescence microscope at  $\times 1000$  magnification. One diagonal of the filter or at least 100 cells were counted.

Ciliate and phytoplankton samples were fixed with 0.2% alkaline Lugol's solution. Fifty milliliters of fixed samples were settled in a sedimentation chamber for 24–48 h and counted according to the Utermöhl technique at  $\times 200$  or  $\times 400$  magnification. Half of the sedimentation chamber or two diagonals (diameter 26 mm) were scanned when counting ciliates and large phytoplankton ( $> 10 \mu\text{m}$  in size). Small phytoplankton ( $< 10 \mu\text{m}$  in size) were counted at  $\times 400$  magnification in two diagonals.

Chl *a* was analyzed in a Perkin Elmer LS 30 fluorometer (433 nm excitation wavelength and 674 nm emission wavelength). One hundred milliliters of water was filtered ( $\leq 100$  mm Hg) onto 25-mm Whatman GF/F filters. Chl *a* was extracted in 10 mL 95% ethanol for 24 h at room temperature in darkness.

Primary productivity was measured using the  $^{14}\text{C}$  technique (Gargas 1975). Triplicate dark and light 9-mL samples were incubated in acid-washed polycarbonate bottles with  $26.8 \times 10^3$  Bq ( $3.7 \times 10^6$  Bq  $\text{mmol}^{-1}$ ) sodium ( $^{14}\text{C}$ ) bicarbonate for 4 h. Samples were then poured into glass scintillation bottles, acidified with 300  $\mu\text{L}$  of 6 mol  $\text{L}^{-1}$  HCl, and bubbled for 30 min to remove the excess sodium ( $^{14}\text{C}$ ) bicarbonate. After adding scintillation cocktail (Optiphase, Hi-Safe 3), samples were analyzed in a Beckman 6500 scintillation counter. Net primary productivity was calculated according to Gargas (1975).

Bacterial productivity was measured using the [ $^3\text{H}$ -methyl]-thymidine technique (Fuhrman and Azam 1982). Triplicate 1-mL samples were incubated for 60 min with  $74 \times 10^3$  Bq ( $3.2 \times 10^{12}$  Bq  $\text{mmol}^{-1}$ ) [ $^3\text{H}$ -methyl]-thymidine. The incubations were stopped by adding 100  $\mu\text{L}$  ice-cold 50% trichloroacetic acid (TCA). Nonincorporated thymidine was washed away with ice-cold 5% TCA and centrifugation. Triplicate controls were pre-killed with 100  $\mu\text{L}$  50% TCA and treated as described above. Incorporated thymidine was measured with a Beckman 6500 scintillation counter. A conversion factor of  $1.5 \times 10^{18}$  cells per mole of incorporated thymidine was used to calculate cell productivity (Wikner and Hagström 1999).

Four-liter samples were taken for measurement of mesozooplankton abundance. Mesozooplankton were collected on a 90- $\mu\text{m}$  mesh and preserved with 0.2% alkaline Lugol's solution. An inverted microscope was used to identify, count, and measure the length of 10 individuals (all, if fewer were collected) of each taxa. Copepods were classified into two groups: nauplii and copepodites. Lengths were transformed to body mass using length–mass regressions (Dumont et al. 1975; Hernroth 1985) and assuming 5% carbon content of wet mass. Mesozooplankton productivity was estimated from measured abundance, body mass, and literature data on development time at 15°C (Heinle and Flemer 1975). Since the mesozooplankton community was dominated by copepods, all other groups were ignored in the calculations. The calculation of mesozooplankton productivity was based on the number and weight of nauplii produced during each time interval and the weight increase of the surviving nauplii developed to copepodites at each time interval, according to the following equation:

$$\text{MZ}_p = \frac{(n_1 \times w_n) + (n_0 \times [1 - (m/100)] \times [w_c - w_n])}{t_1 - t_0} \quad (1)$$

Mesozooplankton net productivity ( $\text{MZ}_p$ ) is expressed in  $\mu\text{g C L}^{-1} \text{d}^{-1}$ , where  $n_1$  = number of nauplii  $\text{L}^{-1}$  at time  $t_1$ ,  $w_n$  = nauplii weight in  $\mu\text{g C}$  per individual (Hernroth 1985),  $n_0$  = number of nauplii  $\text{L}^{-1}$  at time  $t_0$ , and  $w_c$  = mean copepodite weight in  $\mu\text{g C}$  per individual from this study. Mesozooplankton mortality ( $m$ ) was estimated by the

following equation:

$$m(\%) = \frac{(c_{\text{est}} + c_0 - c_1)}{(c_{\text{est}} + c_0)} \times 100 \quad (2)$$

where  $c_{\text{est}}$  = estimated number of nauplii  $\text{L}^{-1}$  that have become copepodites, assuming no mortality, and according to development time from literature;  $c_0$  = measured number of copepodites at  $t_0$ ; and  $c_1$  = actual number of copepodites present at  $t_1$ . The sampling intervals were set so that nauplii at the start of each time interval had all developed to copepodites at the end of the interval, and all nauplii occurring at the end of each time interval were produced from eggs during that period. The productivity for each interval  $t_0$ – $t_1$  is displayed as the midpoint between  $t_0$  and  $t_1$  in the graph.

FWE was defined as mesozooplankton productivity per basal productivity and calculated as

$$\text{FWE} = \frac{\text{MZ}_p}{\text{PP} + \text{BP}} \quad (3)$$

Mesozooplankton net productivity ( $\text{MZ}_p$ ), net primary productivity (PP), and net bacterial productivity (BP) were calculated in  $\mu\text{g C L}^{-1} \text{d}^{-1}$ .

The isotopic signals of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in mesozooplankton were analyzed at the end of the experiment. All mesozooplankton in the mesocosms were collected on a 90- $\mu\text{m}$  mesh and immediately frozen at  $-20^\circ\text{C}$ . Before analysis, the mesozooplankton were washed with Milli-Q water and subsequently collected on a 200- $\mu\text{m}$  mesh to get rid of microorganisms and most phytoplankton. Larger filaments of phytoplankton were removed by using a Pasteur pipette. The clean mesozooplankton fraction larger than 200  $\mu\text{m}$  was finally collected on a GF/F filter and dried at  $60^\circ\text{C}$  for 24 h. Analyses of the solid samples were performed using a Europa Scientific carbon and nitrogen analyzer connected to a Europa 20-20 stable isotope analyzer. Nitrogen and carbon isotopic compositions are expressed in per mil (‰) deviations from a standard. For example,

$$\delta^{13}\text{C} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ where } R = \frac{^{13}\text{C}}{^{12}\text{C}} \quad (4)$$

The standard deviation (SD) of both carbon and nitrogen standard samples was 0.2‰. Carbon and nitrogen content of mesozooplankton was analyzed by collecting 10–12 individuals from each treatment on glass-fiber filters (Whatman GF/F precombusted for 4 h at  $450^\circ\text{C}$ ). Analysis was performed with a Carlo Erba model 1108 high-temperature combustion elemental analyzer, using standard procedures and a combustion temperature of  $1030^\circ\text{C}$ . Acetanilide was used for standardization, and results were corrected for blank filter carbon content.

The fraction of glucose, i.e., bacterial carbon, assimilated by mesozooplankton ( $\text{MZ}_{\text{glucose}}$ ) in the CNP treatment was estimated by assuming that the mesozooplankton carbon signal in the CNP treatment ( $\text{MZ}_{\text{CNP}}$ ) would have been similar to the NP treatment ( $\text{MZ}_{\text{NP}}$ ) if there were no

assimilation of glucose:

$$MZ_{\text{glucose}}(\%) = \frac{(MZ_{\text{CNP}} - MZ_{\text{NP}})}{([\text{Glu} + t_l \times F] - MZ_{\text{NP}})} \times 100 \quad (5)$$

Glu is the measured carbon isotopic signal of glucose. This carbon was considered to pass through one to three trophic links ( $t_l$ ) before reaching the mesozooplankton, and the fractionation by bacteria was assumed to be negligible. The trophic fractionation of carbon ( $F$ ) was set to 0.39‰ according to Post (2002). The influence of differences in the fractionation factor was tested by assuming  $F$  values ranging from  $-0.91$  to  $1.69$ ‰ (Post 2002).

Since respiration was expected to differ in the two treatments, oxygen consumption was estimated by measuring oxygen concentration before and after incubating 130 mL water in the dark for 24 h. The Winkler titration method was used (Swedish Standard SS-EN 25 813).

Concentrations of inorganic nutrients (molybdate reactive phosphorus, nitrate, nitrite, ammonium, and silicate) were analyzed in a Braan and Luebbe TRAACS 800 autoanalyzer (Grasshoff et al. 1983). Fifty milliliters of water were filtered through  $0.2\text{-}\mu\text{m}$  cellulose-acetate filters (Gelman Supor) and kept frozen until analysis. Concentration of DOC was measured in a high-temperature carbon analyzer (Shimadzu TOC-5000). Approximately 10 mL of water was filtered through  $0.2\text{-}\mu\text{m}$  pore size filters (Gelman Supor) and acidified with  $100\ \mu\text{L}$  of  $2\ \text{mol L}^{-1}$  HCl before analysis. All materials in contact with the samples, including the filters and filter units, were acid washed with  $1.2\ \text{mol L}^{-1}$  HCl and rinsed with Milli-Q water prior to use.

*Statistical analyses*—Differences between treatments of most parameters were tested using repeated measures of analysis of variance (ANOVA) in SPSS 11 for Windows. Before analysis, the data were checked for any violation of the assumptions for repeated measures of ANOVA. Carbon and nitrogen content in mesozooplankton and food web efficiency were analyzed by using the  $t$ -test. Regression analysis was used to explain oxygen consumption data. Independent factors for oxygen consumption were bacterial productivity, primary productivity, and mesozooplankton productivity.

## Results

The addition of glucose caused an increase in bacterial growth and biomass concentration. In the CNP treatment the bacterial productivity averaged 13 times higher than in the NP treatment (Fig. 1). The bacterial biomass was 2.8 times higher in the CNP treatment (Fig. 2, repeated measures of ANOVA,  $F_{1,6} = 62.986$ ,  $p < 0.001$ ). The reduction in light and higher carbon concentration reduced the primary productivity from a mean for the whole period of  $17\ \mu\text{g L}^{-1}\ \text{d}^{-1}$  in the NP treatment to  $1.7\ \mu\text{g L}^{-1}\ \text{d}^{-1}$  in the CNP treatment (Fig. 1). The total productivity averaged three times higher in the CNP treatment than in the NP treatment (repeated measures of ANOVA,  $F_{1,6} = 31.062$ ,  $p = 0.001$ ). The Chl  $a$  was significantly lower in the

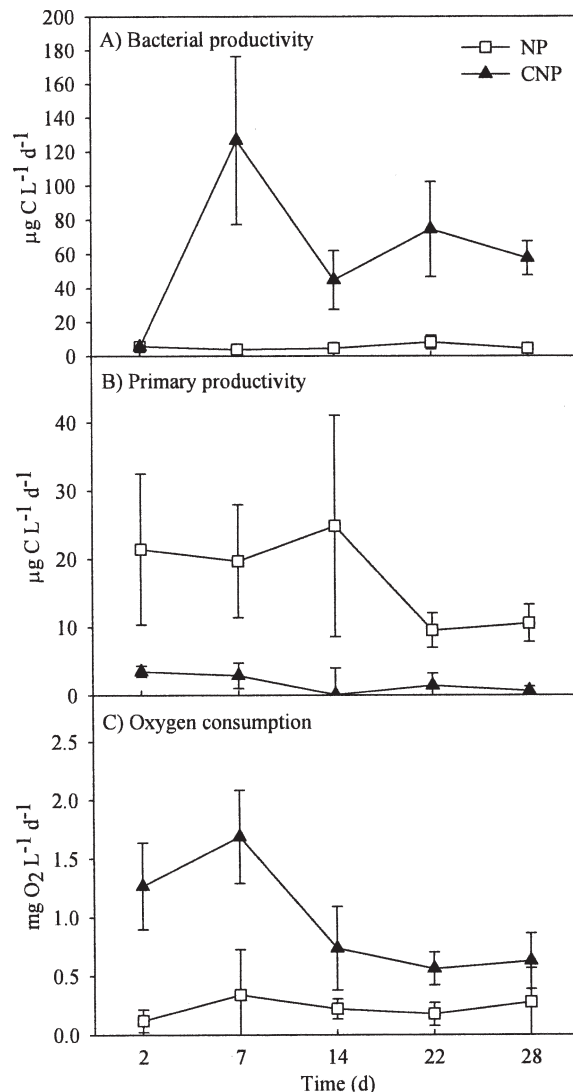


Fig. 1. (A) Bacterial and (B) primary productivity, and (C) oxygen consumption in the NP and CNP treatments. Bars denote  $\pm 1$  SD.

CNP treatment than in the NP treatment (Fig. 2, repeated measures of ANOVA,  $F_{1,6} = 155.69$ ,  $p < 0.001$ ). The oxygen consumption averaged  $\sim 5$  times higher in the CNP treatment (Fig. 1; repeated measures of ANOVA,  $F_{1,6} = 43.914$ ,  $p < 0.001$ ), and the mesozooplankton productivity per unit of oxygen consumption was 30 times lower in this treatment compared to the NP treatment (data not shown). The increased oxygen consumption in the CNP treatment was to a high degree explained by increased bacterial productivity ( $r^2 = 0.53$ ,  $t = 1.30$ ,  $df = 30$ ,  $p < 0.001$ ).

Phosphate and nitrogen were in excess and increased steadily during the experiment (Fig. 3). The concentration of dissolved inorganic phosphate and nitrogen was higher in the NP treatment than in the CNP treatment (repeated measures of ANOVA,  $F_{1,6} = 7.234$ ,  $p = 0.036$ ; and  $F_{1,6} = 18.028$ ,  $p = 0.005$ , respectively). DOC was relatively constant over the course of the experiment but higher in the CNP compared to the NP treatment (Fig. 3, repeated measures of ANOVA,  $F_{1,6} = 293.207$ ,  $p < 0.001$ ).

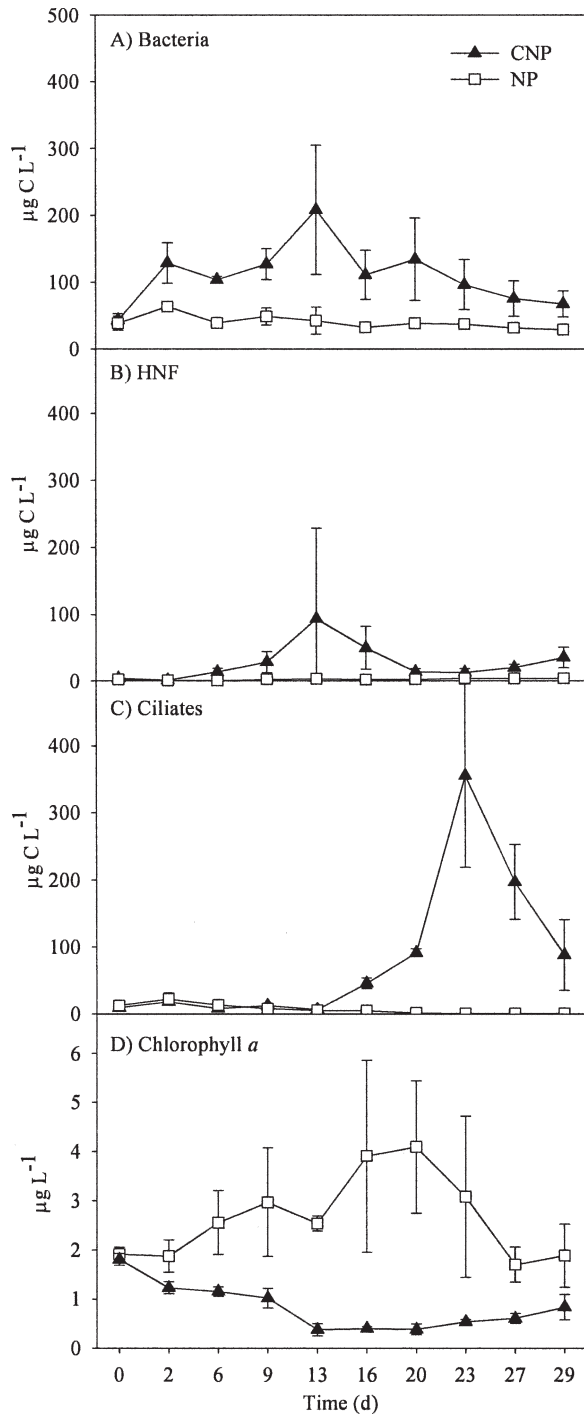


Fig. 2. (A) Bacteria, (B) heterotrophic nanoflagellates (HNF), (C) ciliates, and (D) Chl *a* concentrations in the NP and CNP treatments. Bars denote  $\pm 1$  SD.

Dissolved silicate decreased over the course of the experiment in both treatments, but this decrease was more rapid in the NP treatment (Fig. 3).

High bacterial productivity in the CNP treatment promoted heterotrophic protozoa, flagellates, and ciliates (Fig. 2). The biomass of heterotrophic flagellates averaged 10 times higher in the CNP treatment than in the NP treatment (repeated measures of ANOVA,  $F_{1,6} = 19.846$ ,  $p$

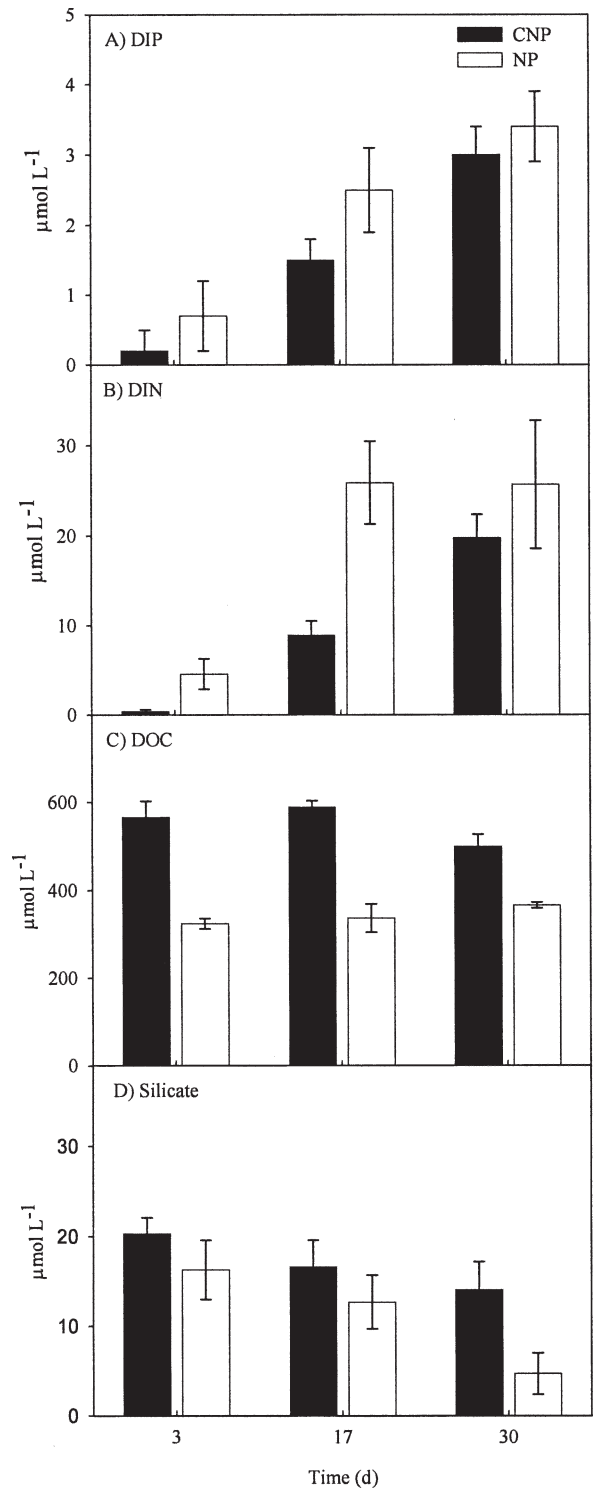


Fig. 3. (A) Dissolved inorganic phosphorus (DIP), (B) dissolved inorganic nitrogen (DIN), and (C) dissolved inorganic carbon (DOC) concentrations in the NP and CNP treatments sampled at the beginning (day 3), middle (day 17), and end (day 30) of the experiment. Bars denote  $\pm 1$  SD. DIP =  $\text{PO}_4^{3-}$ , DIN =  $\text{NO}_2^- + \text{NO}_3^{2-} + \text{NH}_4^+$ .

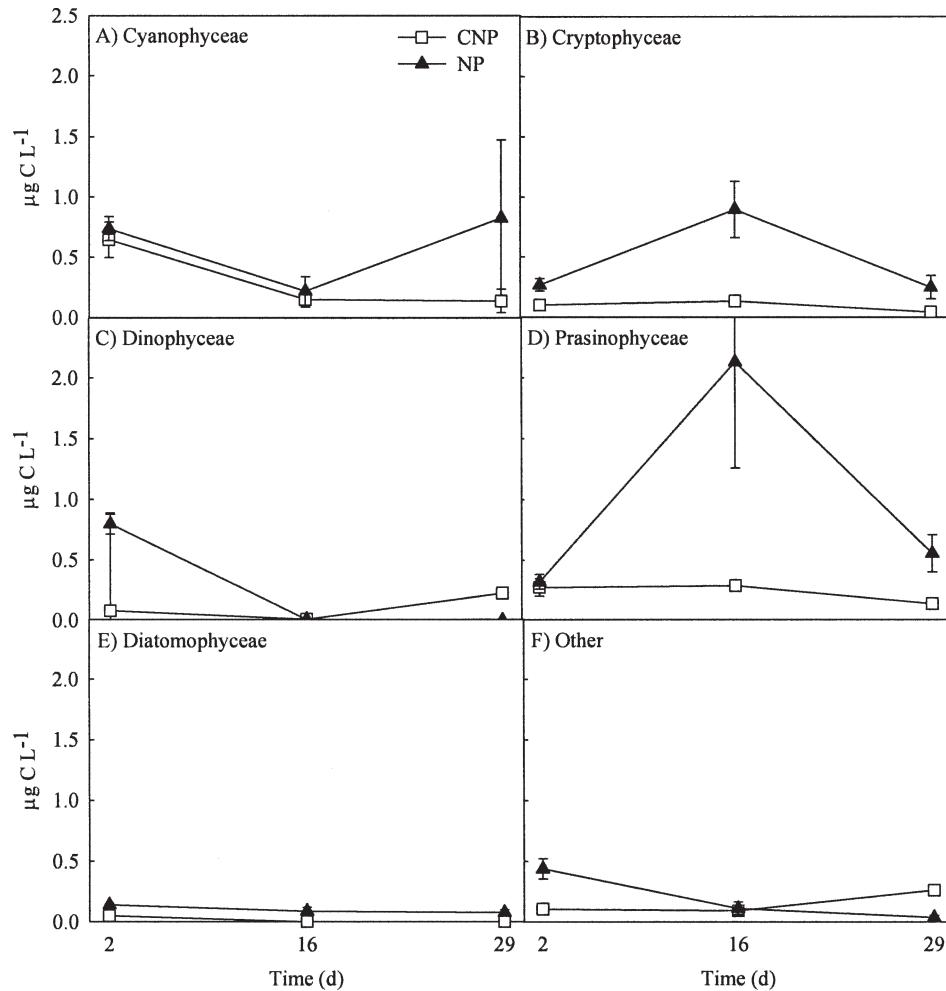


Fig. 4. Biomass of dominating phytoplankton classes at the beginning (day 2), middle (day 16), and end (day 29) of the experiment in the NP and CNP treatments. Other includes the classes Chlorophyceae, Chrysophyceae, Euglenophyceae, and Prymnesiophyceae. Bars denote  $\pm 1$  standard error.

= 0.004). The ciliates peaked after 3 weeks of incubation in the CNP treatment. This peak occurred about 1 week after a peak in flagellate biomass (Fig. 2). There were almost no ciliates in the NP treatment during the second half of the experiment. At the start of the experiment *Balanion* sp., *Lohmaniella* spp., *Mesodinium pulex*, *Strombidium* spp., and *Strobilidium* spp. were common. After 1 week of incubation the ciliate biomass was totally dominated (90%) by members of the genera *Strobilidium* and *Lohmaniella*. An autotrophic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*) also occurred in both treatments throughout the experiment, although constituting only 2% of the total ciliate biomass. Ciliate carbon concentration was 62 times higher in the CNP treatment than in the NP treatment during the second half of the experiment (repeated measures of ANOVA,  $F_{1,6} = 50.326$ ,  $p = 0.001$ ).

At the start of the experiment the phytoplankton biomass was composed of 35% cyanobacteria, 32% dinoflagellates, and 13% prasinophytes. Cryptophytes constituted only 7% of the total biomass. Throughout the experiment there were significantly more chryptophytes,

prasinophytes, and diatoms in the NP treatment (Fig. 4, repeated measures of ANOVA,  $F_{1,6} = 16.478$ ,  $p = 0.007$ ;  $F_{1,6} = 7.064$ ,  $p = 0.038$ ; and  $F_{1,6} = 48.776$ ,  $p < 0.001$ , respectively). Especially the cryptophytes *Plagioselmis prolonga*, *Teleaulax acuta*, *Teleaulax amphioxeia*, and *Hemiselmis virescens* and the prasinophyte *Pyramimonas* sp. were very abundant with concentrations up to 2000 cells mL<sup>-1</sup>. At the end of the incubation, colony-forming or filamentous cyanobacteria together with cryptophytes and prasinophytes dominated the phytoplankton community in the NP treatment, while (athecate) mixotrophic or strict heterotrophic dinoflagellates, e.g., *Gymnodinium* spp. and *Amphidinium* sp., dominated in the CNP treatment (Fig. 4). In the NP treatment the dinoflagellates were initially very common but decreased to very low biomass values during the time course of the experiment (Fig. 4).

The average biomass of mesozooplankton was eight times higher in the NP treatment than in the CNP treatment during the second half of the experiment (Fig. 5; repeated measures of ANOVA,  $F_{1,6} = 22.299$ ,  $p$

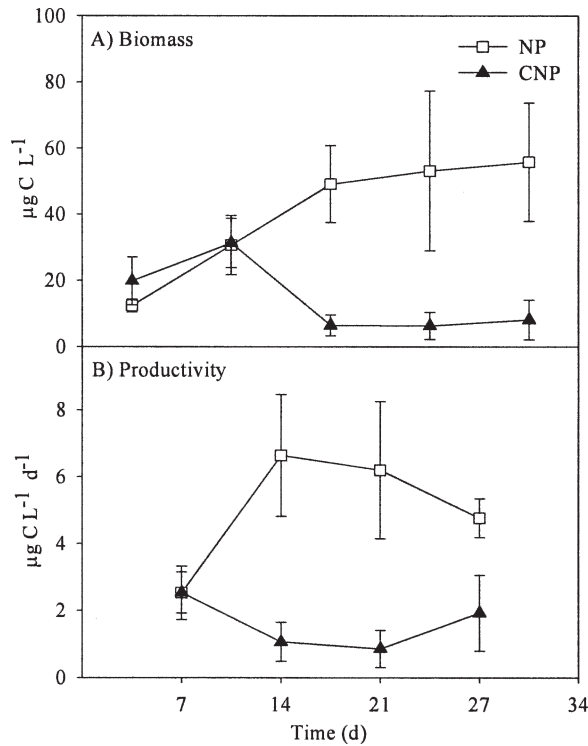


Fig. 5. (A) Mesozooplankton biomass and (B) productivity during the experiment in the NP and CNP treatments. Bars represent  $\pm 1$  SD.

= 0.003). Copepods, especially *Eurytemora affinis*, dominated the mesozooplankton assemblage in both treatments throughout the experiment. They averaged  $45 \pm 30$  copepodites  $L^{-1}$  in the NP treatment and  $11 \pm 7 L^{-1}$  in the CNP treatment. One cladoceran species, *Bosmina* sp., was observed in both treatments, but it was not abundant ( $<3$  individuals  $L^{-1}$ ). Rotifers, *Keratella cochlearis* and *Keratella quadrata*, were abundant ( $71$  individuals  $L^{-1}$ ) at the start of the experiment in both treatments, although constituting less than 0.5% of the total mesozooplankton biomass. The rotifers were not observed after 1 week of incubation (data not shown). The mesozooplankton productivity was similar in both treatments during the first week of incubation (Fig. 5). After that, productivity averaged five times higher in the NP treatment than in the CNP treatment (repeated measures of ANOVA,  $F_{1,6} = 37.387$ ,  $p = 0.001$ ). The mortality of mesozooplankton was about 40% in both treatments during the experiment, except during the second week, when the mortality in the CNP treatment was  $>90\%$ . The mesozooplankton (mainly copepodites) did not seem to be starved in the CNP treatment because there was no significant difference in the average size and carbon content between them and individuals in the NP treatment (repeated measures of ANOVA,  $F_{1,6} = 0.217$ ,  $p = 0.658$ ). However, the C:N molar ratio of the mesozooplankton was higher in the phytoplankton-based food web than in the bacteria-based food web, 6.3 and 5.2, respectively ( $t = 3.14$ ,  $df = 3$ ,  $p = 0.05$ ).

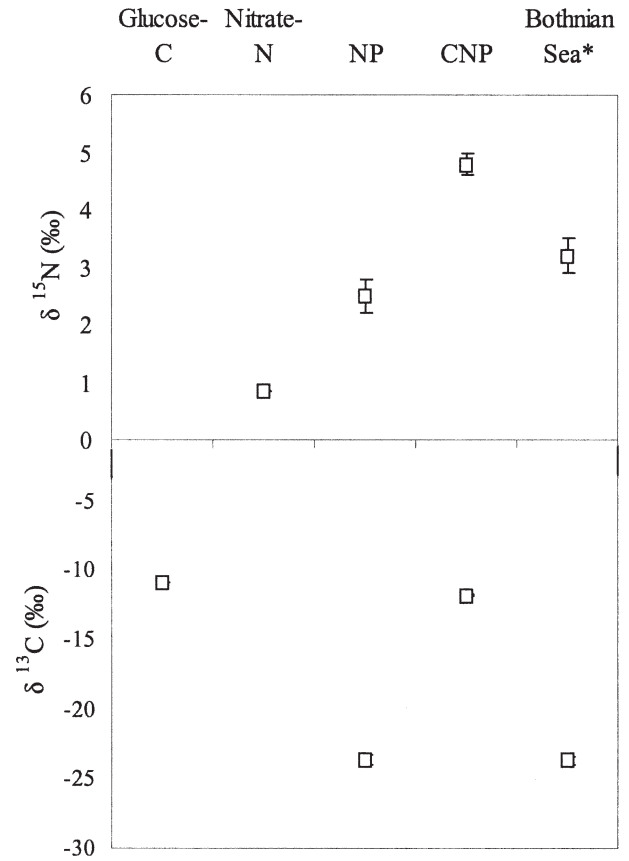


Fig. 6. Isotopic ratio of  $\delta^{13}C$  and  $\delta^{15}N$  in added nutrients (glucose, C; nitrate, N), mesozooplankton in the NP and CNP treatments, and in mesozooplankton sampled in the northern Baltic Sea (Bothnian Sea). Bars represent  $\pm 1$  SD (standard deviation of carbon signal not visible). \*Rolf and Elmgren (2000).

The mesozooplankton  $\delta^{13}C$  signal in the CNP treatment showed that the carbon mainly was of bacterial origin, i.e., similar to the carbon signal of the added glucose (Fig. 6). Assuming a carbon fractionation factor of 0.39‰, the contribution of glucose to mesozooplankton in the CNP treatment would have been 85–90%, depending on the number of trophic links. When different carbon fractionation factors were tested, the contribution of glucose ranged from 66% to 100%. In the NP treatment, the  $\delta^{13}C$  value of mesozooplankton was similar to values from an adjacent station in the northern Baltic Sea (Fig. 6). The  $\delta^{15}N$  signal indicated that the bacteria-based food web was longer than the phytoplankton-based food web. The added nitrate had a  $\delta^{15}N$  value of 0.8‰, and the mesozooplankton in the NP and CNP treatments had mean values of 2.5‰ and 4.8‰, respectively (Fig. 6). The biomass of mesozooplankton per resource productivity was 20-fold higher where phytoplankton constituted the basal resource (data not shown,  $t = 8.30$ ,  $df = 6$ ,  $p < 0.001$ ). The food web efficiency (mesozooplankton productivity per unit of resource productivity) averaged  $22\% \pm 8\%$  in the NP treatment and  $2\% \pm 1\%$  in the CNP treatment (Fig. 7), i.e., the food web efficiency was 11-fold higher in the phytoplankton-based food web.

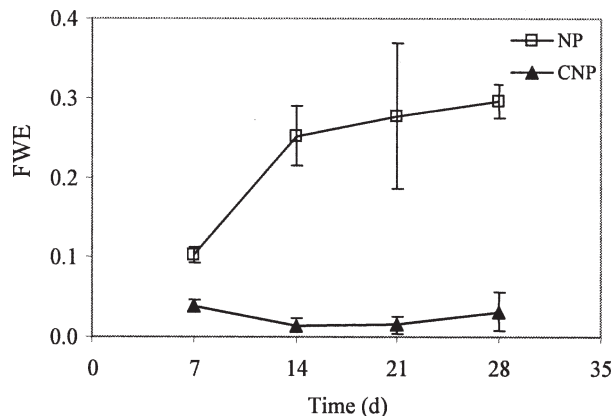


Fig. 7. Estimated food web efficiency (FWE) in the NP and CNP treatments during the experiment. Bars represent  $\pm 1$  SD.

## Discussion

The results showed that the phytoplankton-based food web was much more efficient than the bacteria-based web. During the second half of the experiment, the food web efficiency in the CNP treatment was only 9% of that in the NP treatment. The lower food web efficiency in the CNP treatment could be explained by additional trophic levels. The abundance of heterotrophic flagellates and ciliates was 10 and 12 times higher in the CNP treatment, indicating that the carbon was channeled through these components of the food web instead of being transferred directly to mesozooplankton (Fig. 8). The trophic position of mesozooplankton was estimated by stable isotope analysis (Post 2002). It is generally assumed that there is a  $\delta^{15}\text{N}$  enrichment of 2–5‰ per trophic level (Post 2002). In the Baltic Sea, the trophic distance between phytoplankton and mesozooplankton has been estimated to vary seasonally from 1.7‰ to 5.5‰ (Rolf 2000). We found a difference of 2.3‰ between mesozooplankton in the NP and in the CNP treatment. If we employ the estimated range in trophic distance, 1.7–5.5‰ (Rolf 2000), the food web should have been 0.4–1.5 trophic levels longer in the CNP treatment. Furthermore, the difference in number of trophic levels between the treatments can be calculated by using general gross growth efficiencies (GGE), together with observed difference in food web efficiency between the two treatments (11-fold):

$$(\text{GGE})^X = 1/11$$

where  $X$  is the number of extra trophic levels in the CNP treatment. Protozoan and metazoan GGE values of 20–40% can be used in this calculation (Straile 1997; Båmstedt et al. 1999), which would correspond to 1.5–2.6 extra trophic levels. Lower GGE causes fewer additional trophic levels. By combining the different calculations, we conclude that the bacteria-based food web on average would have been 1.5 trophic levels longer than the phytoplankton-based food web.

These results are further supported by the stable isotope analyses of mesozooplankton carbon. Of mesozooplankton

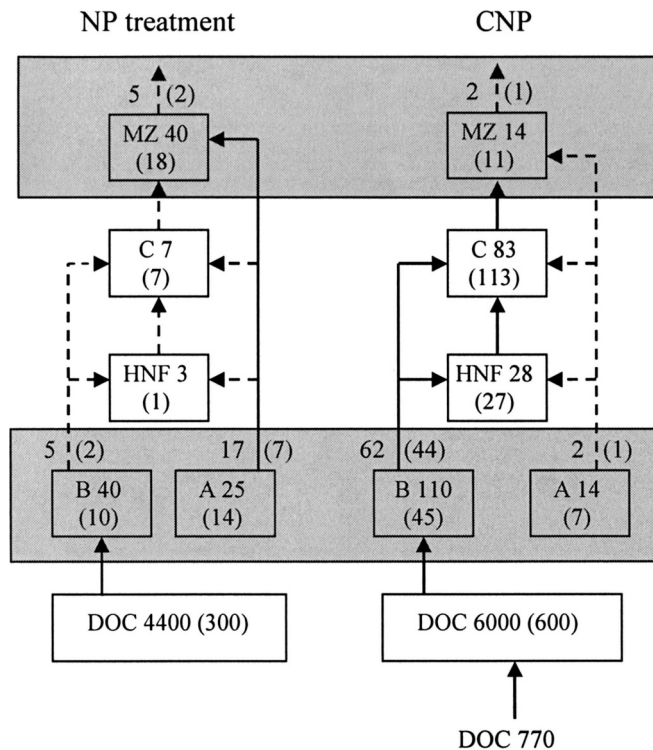


Fig. 8. Simplified model of the food web structure in the NP and CNP treatment. Average carbon biomasses of different functional groups are presented within boxes ( $\mu\text{g C L}^{-1}$ ). Productivity of bacteria, phytoplankton, and mesozooplankton are presented above biomass boxes ( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ). Values within parentheses denote 1 SD. Dominant carbon flows, inferred from stable isotope analysis, are marked with solid lines. MZ = mesozooplankton, C = ciliates, B = bacteria, HNF = heterotrophic nanoflagellates, A = algae, B = bacteria, DOC = dissolved organic carbon.

carbon in the CNP treatment, 85% to 90% originated from added glucose. Because mesozooplankton are not specialists in direct uptake of glucose, they probably received it via bacteria and microbial food web transfer. If we assume a linear carbon flow from the added glucose ( $770 \mu\text{g C L}^{-1} \text{d}^{-1}$ ) through bacteria, heterotrophic nanoflagellates, and ciliates, ending up in copepod productivity ( $2 \mu\text{g C L}^{-1} \text{d}^{-1}$ ), the average gross growth efficiency would be 23% assuming 100% copepod productivity from added C, or 22% if we assume that 85% of the copepod productivity was based on this pathway. The copepod species *E. affinis*, which dominated the mesozooplankton assemblage in our experiment, feeds well on both heterotrophic and autotrophic nanoplankton and microplankton (Gasparini and Castel 1997; Merrell and Stoecker 1998; Finlay and Roff 2004). The copepod may even prefer feeding on heterotrophic organisms.

Further proof of dominating microbial transfer in the CNP treatment is that the phytoplankton productivity was too small to sustain the mesozooplankton productivity. If we assume that the mesozooplankton had a gross growth efficiency of 33% (e.g., Kiørboe et al. 1985; Båmstedt et al. 1999), the phytoplankton could explain up to 30% of mesozooplankton productivity, leaving 70% or more of the

productivity based on ciliates and heterotrophic nanoflagellates. It is less likely that bacteria directly were a significant component in the copepod diet as a result of the very low capture efficiency of picoplankton (Finlay and Roff 2004).

It is likely that omnivory was high in the bacteria-based food web. By assuming an instantaneous ciliate growth rate of 0.2–0.5 d<sup>-1</sup> (e.g., Gismervik et al. 2002), a ciliate productivity of 17–41  $\mu\text{g C L}^{-1} \text{d}^{-1}$  (0.2–0.5 times the biomass of 83  $\mu\text{g C L}^{-1} \text{d}^{-1}$  would be expected (Fig. 8). The FWE would thus have been 27–64% from bacteria–phytoplankton to ciliates in the CNP treatment. The high FWE values of the hypothetically truncated food web indicate significant omnivory by the ciliates feeding directly on bacteria. Also, copepods may efficiently have grazed on both the flagellate and ciliate trophic levels (Gasparini and Castel 1997; Merrell and Stoecker 1998; Finlay and Roff 2004). Omnivory in the CNP treatment thus explains why the food web was not fully two trophic levels longer in the CNP treatment compared to the NP treatment.

It is possible that the relatively short length of the experiment led to an underestimation of the food web efficiency from the lowest trophic level to mesozooplankton in the CNP treatment. Ciliates and dinoflagellates peaked during the last week of the experiment, and these protists would have been high-quality food for the copepods (Gasparini and Castel 1997; Vargas and Gonzales 2004). Thus, it is possible that the mesozooplankton did not have time to respond to the increased amount of large protozoa. However, even if the food web efficiency was underestimated, the longer pathway in this treatment would still generate a decreased FWE also on long-term basis. The estimated food web efficiency in the NP treatment of 22% is within the range of the GGE reported for copepods (e.g., Kiørboe et al. 1985; Båmstedt et al. 1999). This high efficiency indicates a direct carbon transfer from phytoplankton to copepods (Fig. 8).

Earlier studies in both marine and freshwater systems show that the transfer efficiency from both phytoplankton and bacteria to mesozooplankton can be low, 0.1–1% (Ducklow et al. 1986; Koshikawa et al. 1999; Havens et al. 2000). The food web efficiency in our bacteria-based treatment was comparable to these earlier studies (2%), but the efficiency of the phytoplankton-based food web was higher (22%). The low carbon flux from phytoplankton to mesozooplankton observed in earlier studies has been explained by low edibility of dominating algae and the features of the mesozooplankton (Koshikawa et al. 1999; Havens et al. 2000). Koshikawa et al. (1999) found a higher carbon transfer when appendicularians were more abundant than copepods. Autotrophic nanoflagellates dominated the phytoplankton assemblage in the NP treatment in our experiment. These algae are excellent food items for the dominating copepod *E. affinis* (Gasparini and Castel 1997). Since nanoplankton dominate the phytoplankton biomass in the studied area during the summer (Andersson et al. 1996), the measured food web efficiency in the NP treatment seems to be relevant.

There is a possibility that food quality (chemical composition), rather than food quantity, may have limited

zooplankton growth (Sterner and Hessen 1994). We did not measure the elementary composition of the resource in the experiment, but in general bacteria have a lower molar C:N ratio (4–5) than phytoplankton (6.63) (Fagerbakke et al. 1996; Makino et al. 2003; Redfield ratio). This seems to have been reflected in the consumers. In the bacteria-based food web, the mesozooplankton C:N ratio was lower than in the phytoplankton-based web. In the Baltic Sea, the C:N ratio of copepods has been shown to be stable, around 5–6 (Koski 1999; Pertola et al. 2002). However, in experiments, the C:N ratio in copepods has been shown to increase with increasing food supply (Koski 1999). It may therefore be argued that mesozooplankton in the CNP treatment was more starved than in the NP treatment. In that case the difference in resource quality between the two treatments may have resulted in lower mesozooplankton productivity. However, the size and carbon content of copepodites were not lower in the CNP treatment, indicating that there was no severe starvation.

The ratio of bacterial to primary productivity in the NP treatment was comparable to measures from the northern Baltic Sea (Sandberg et al. 2004). The average primary productivity of 18  $\mu\text{g C L}^{-1} \text{d}^{-1}$  and bacterial productivity of 5  $\mu\text{g C L}^{-1} \text{d}^{-1}$  is representative for the season and the area (Andersson et al. 1994; Johansson et al. 2004). In the CNP treatment, the bacterial productivity averaged 61  $\mu\text{g C L}^{-1} \text{d}^{-1}$ , which is about five times higher than maximum rates measured at coastal stations in the area (Andersson et al. 1994). The bacterial contribution in the NP treatment (26%) was similar to values estimated from coastal waters. The yearly contribution of bacteria to total productivity in the northern Baltic Sea is 27% (Sandberg et al. 2004). The bacterial contribution in the CNP treatment was 91%, which is comparable to annual values for some unproductive or humic lakes (Karlsson et al. 2001, 2002).

The total productivity was threefold higher in the CNP treatment than in the NP treatment. Higher productivity may result in increased occurrence of inedible prey (Leibold 1989; Steiner 2001) and thereby decreased food web efficiency (Havens et al. 2000; Sommer et al. 2002). In our experiment, we changed the basal resource from phytoplankton to heterotrophic bacteria. Bacteria are known to have different predation defense mechanisms, among which a change in cell size is most frequently acknowledged (Jürgens and Matz 2002). However, bacterial cell volumes in the more productive CNP treatment were 0.1–0.2  $\mu\text{m}^3$  (data not shown), which is within the preferred size range of, e.g., heterotrophic nanoflagellates (González et al., 1990). Hence, the prey in the more productive treatment should have been edible by protozoa at least considering size. The high biomass of both heterotrophic nanoflagellates and ciliates supports this conclusion (cf. Figs 2 and 8).

Ecological theory predicts that increased productivity allows more trophic levels to establish at the top of the food web (Oksanen et al. 1981). The question is then whether reduced productivity in the CNP treatment to one-third of the actual one (i.e., identical with the NP treatment) would have eliminated the mesozooplankton level. Such a food web would eliminate the basis for planktivorous fish and,

thus, significantly diverge from a phytoplankton-based system. If the reduction of basal productivity to one-third of its actual level in the CNP treatment also reduced ciliate productivity proportionally, the latter would range from 6 to 14  $\mu\text{g C L}^{-1} \text{d}^{-1}$ , which should provide sufficient food to maintain the mesozooplankton level. Furthermore, previous results from field studies in the northern Baltic Sea indicate that the food web structure is rather robust to changes in productivity. Berglund et al. (2005) did not find any difference in the food web structure or trophic level control in a gradient with fourfold increase in productivity. We therefore conclude that a reduced basal productivity in the CNP treatment to one-third of its actual level would not change the food web structure.

A possible confounding factor in the experiment was the lower light in the CNP treatment ( $10 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) compared to that in the NP treatment ( $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ). This was performed to give heterotrophic bacteria an advantage over phytoplankton in the competition for nutrients. The light manipulation helped to keep the productivity at similar levels in the NP and the CNP treatment. However, the dominating mesozooplankton group, the calanoid copepods, uses both mechanosensing and chemoreception when capturing food particles (Turner 2004). Hence, they do not need light for efficient grazing. On the contrary, there is a tendency toward more intense feeding in lower light than in high light conditions among marine calanoids (Vijverberg 1989), and they may also capture ciliates in complete darkness (Merrell and Stoecker 1998). During daytime,  $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  corresponds to  $\sim 5 \text{ m}$  depth, and  $10 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  to  $\sim 10 \text{ m}$  depth. These are depths where mesozooplankton occur frequently in the natural system. Therefore, we do not believe that the light variation affected the feeding behavior of the mesozooplankton in the experiment.

The bacterial productivity in the northern Baltic Sea is to a large extent based on ADOC (Sandberg et al. 2004). In a global warming scenario, increased precipitation in northern Europe has been predicted, probably causing an increase in ADOC and nutrient input to marine systems (Bergström et al. 2001). Concomitantly the humic substances in the water will increase, potentially causing the phytoplankton primary productivity to decrease as a result of shading effects. Thus, increased input of organic matter to pelagic systems may result in a shift toward a bacteria-based system. In addition, the increased freshwater transport and mixing with the surface water will probably cause a deepening of the halocline, which in turn will cause a poorer light climate for the phytoplankton. The combined effect of increased ADOC and freshwater transport would thus give bacteria a competitive advantage over phytoplankton. Changes in this direction have been observed after some extremely high freshwater inflows to the Gulf of Bothnia and northern Baltic Sea during the last decade (Andersson and Wikner unpubl. data). The primary productivity has decreased while the bacterial productivity has been stable. Although we did not include planktivorous fish in our study, a logical extrapolation of our results would be that a change from a phytoplankton-based food web toward a bacteria-

based food web also would give considerably lower fish productivity. We find this to be a possible future scenario occurring in ecosystems like the Baltic Sea as a result of the secondary effects of global warming.

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